Arginine deiminase pathway is far more important than urease for acid resistance and intracellular survival in *Laribacter hongkongensis*: a possible result of *arc* gene cassette duplication

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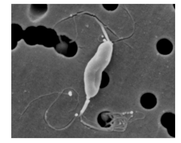

INTRODUCTION & PURPOSE

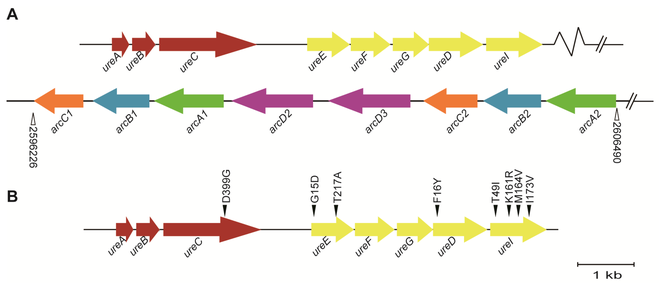

Fig. 1. Genetic organization of urease gene cassette and the two adjacent *arc* gene cassettes in the genome of *L. hongkongensis*.

Laribacter hongkongensis is a Gram-negative, urease-positive bacillus associated with invasive bacteremic infections in liver cirrhosis patients and fish-borne community-acquired gastroenteritis and traveler's diarrhea (1-2). Its mechanisms of acid resistance are unknown. a complete urease cassette and two adjacent *arc* gene cassettes (encoding enzymes of ADI pathway) were found in the genome (3). In this study, we investigated the mechanism for resisting acidic environment in vitro, in macrophages and in a mouse model.

METHODS

- Construction of non-polar deletion mutant strains (urease-negative and ADI- negative mutants);
- In vitro susceptibility of *L. hongkongensis* to acid pH (pH 2 to 6);
- Intracellular survival assays of wild type and mutants in J774 macrophages;
- Survival of *L. hongkongensis* in mouse model.

RESULTS

1. At pH 2 and 3, survival of HLHK9Δ*arcA1*/*arcA2* and HLHK9Δ*ureA*/*arcA1*/*arcA2* were markedly decreased (P<0.001), while HLHK9Δ*ureA* was slightly decreased (P<0.05).

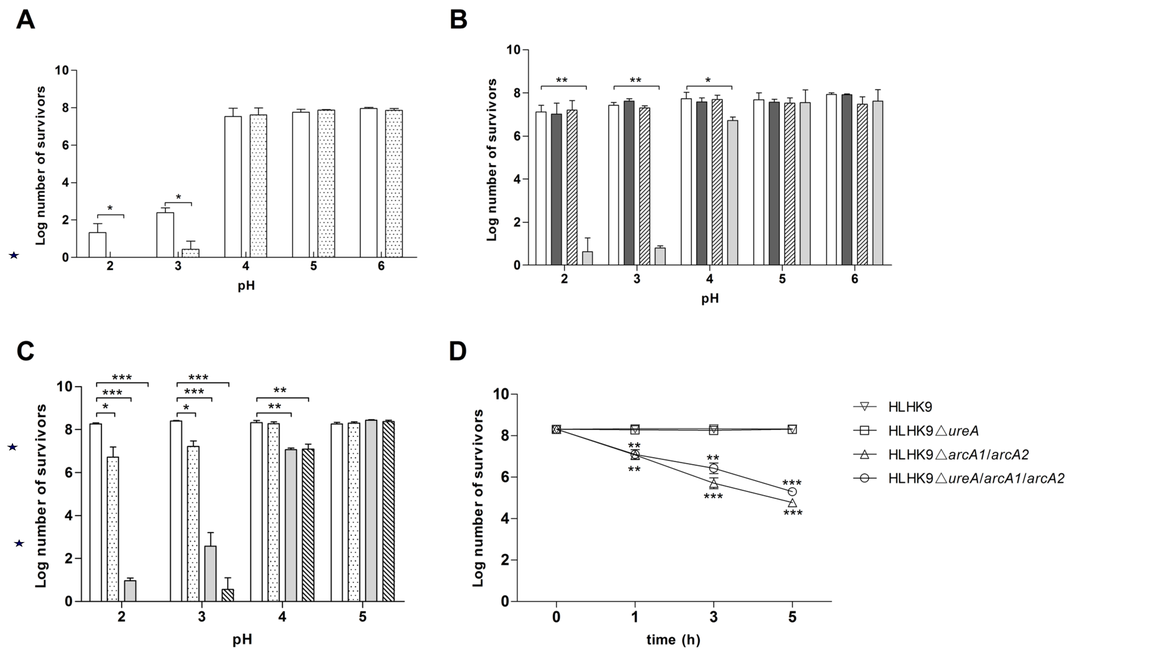

Fig. 2. Survival of wild type *L. hongkongensis* HLHK9 and derivative mutants under acidic conditions. An asterisk indicates a significant difference (*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001).

2. HLHK9Δ*ureA*/*arcA1*/*arcA2* and HLHK9Δ*arcA1*/*arcA2* in macrophages were markedly decreased (P<0.001 and P<0.01 respectively) but that of HLHK9Δ*ureA* was slightly decreased (P<0.05), compared to wild type *L. hongkongensis* HLHK9. Although the mRNA levels of *arcA1*, *arcA2* and *ureA* genes were all significantly increased compared to those at 2 h post infection.

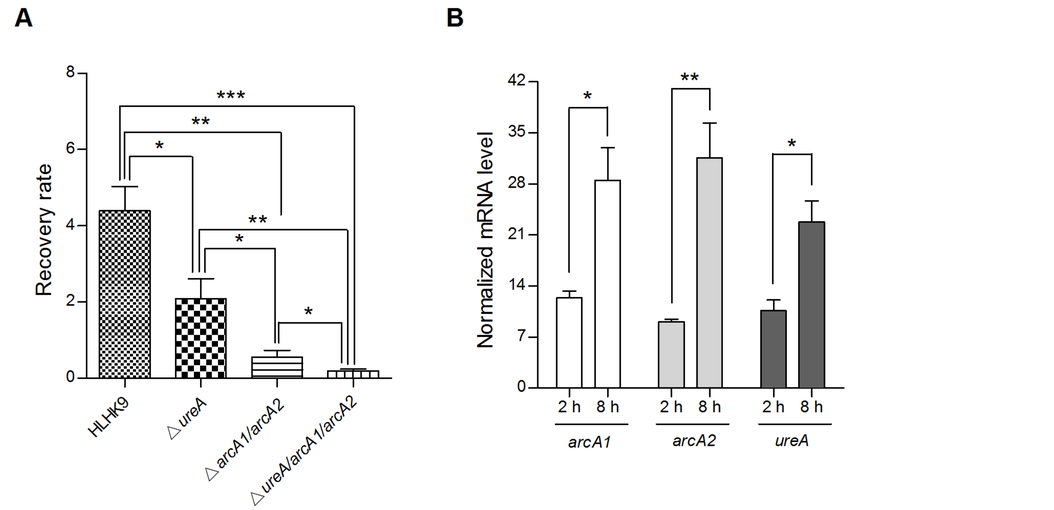

Fig. 3. (A) Recovery rates of wild type *L. hongkongensis* HLHK9, HLHK9Δ*ureA*, HLHK9Δ*arcA1*/*arcA2* and HLHK9Δ*ureA*/*arcA1*/*arcA2* in J774 macrophages. (B) Expression level of ADI genes (*arcA1* and *arcA2*) and *ureA* gene of HLHK9 in macrophages.

3. HLHK9Δ*ureA* exhibited similar survival compared to HLHK9, but survival of HLHK9Δ*arcA1*/*arcA2* and HLHK9Δ*ureA*/*arcA1*/*arcA2* were markedly reduced (P<0.01).

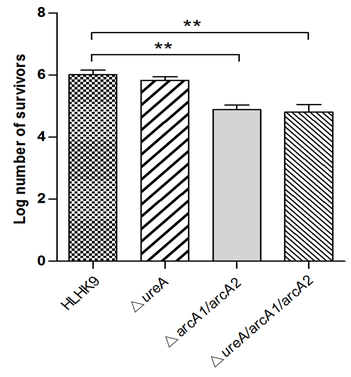

Fig. 4. Survival of wild type *L. hongkongensis* HLHK9 and derivative mutants in a mouse gastric passage model. Comparison of the survival of wild type *L. hongkongensis* HLHK9, HLHK9Δ*ureA*, HLHK9Δ*arcA1*/*arcA2* and HLHK9Δ*ureA*/*arcA1*/*arcA2* after passing through the stomach of mice. Error bars represent means ± SEM of three independent experiments. An asterisk indicates a significant difference.

CONCLUSIONS

- The ADI pathway of *L. hongkongensis* plays a much more important role than the urease in resisting acidic environments;
- Both of ADI pathway and urease system have significant contribution to the replication of *L. hongkongensis* in macrophages; however, the ADI pathway plays a more important role.
- The ADI pathway plays a more significant role than urease in the survival of *L. hongkongensis* under the acidic conditions encountered during passage through the mouse gastric environment.

REFERENCES

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